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# Excessive Transitory Migration of Guppy Populations. III. Analysis of Perception of Swimming Space and a Mirror Effect

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**ABSTRACT**—In an aquarium halved with a slitted septum, a population of guppy put into one compartment moved excessively to the other compartment (the excessive transitory migration, E.T.M.) and gradually approached and equal distribution. It was analyzed using a mirror whether the initial excessive migration and subsequent approach to final balance is performed by the information of real space or visual images of space. If the fish move according to the information by non-visual sense, their migration would be excessive referring to a balance level owing to the volume ratio between compartments. On the other hand, if they behave so due to visual informations, the initial migration and subsequent attainment to final balance would be performed referring to a balance expected from the ratio modified by a mirror image. Experiments showed that the population approached a balance expected from visual information, not that from real space. The information of the density of fish in the recovery to final balance was discussed in relation to the perception of real space or visual image of space.

## INTRODUCTION

In an aquarium with a slitted septum, guppies put in one side move to the other side excessively, then they gradually reach an equal distribution. As they migrated from the clean water to polluted water with their excretions, the excessive migration could not be subjected to avoiding the substances [1]. Another supposition that they might exhibit the excessive migration due to their possible schooling tendency was also rejected by detailed analyses of both the distribution of runs of slit-passing and the oscillographic records [2]. We stated previously [1] that the avoidance from the pressure of excessive population might be one of the causes of excessive migration in the initial phase, because the fish which had been confined in a smaller space moved away more promptly.

In our recent trials, when an aquarium was unequally divided, the guppy population behaved referring to a balanced state expected from the volume ratio between compartments. How does the guppy perceive the balance state? If the fish behave referring to an apparent extension of swimming space, when a mirror is placed in one compartment, they will respond to the space modified by the mirror, i.e. they will be in more number in the compartment with the mirror. If the fish perceive the real swimming space, they will react irrespective of the mirror.

In this paper we studied using a mirror the guppy's space perception by observing the initial migration and the subsequent recovery in an equal- and an unequal-divided tanks.

## MATERIALS AND METHODS

Adult female guppies *Poecilia reticulata* used were of 2.8±0.4 cm in total length, bred in our laboratory. Males were not used, since populations of uniform individuals could not be easily obtained because of a great variation in body-to-tail ratio. The guppies were kept in a white-graveled and plant-rooted aquarium. Several days

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before the experiments, populations of 40 fish were isolated from the aquarium and placed in rearing tanks (45×25×25 cm deep). Water temperature was maintained within a range between 23.0 and 27.0°C. An experimental tank (60 ×25×25 cm deep) was halved with a white plastic septum having a vertical slit 25×2 cm. This size was large enough for the passage of the fish. The slit was closed with a plastic plate until the onset of the experiment. White papers were attached on the tank walls excepting the frontal wall for observation.

One group of 40 fish were introduced into the left compartment of an experimental tank. After acclimation to the tank for 15 min the plate was quietly pulled up. The number of fish passing through the slit from the left compartment to the right one and *vice versa* was recorded for 30 min. After the experiment, this data was used to calculate the number of fish in each compartment during 1-minute intervals. In Section 2, a mirror or a transparent glass plate was used.

Whether the migration to the other compartment is excessive or not was tested by the Wilcoxon one-sample signed-ranks test [3] using the median value of 10 data at each minute. In Section 1, when the difference between the minimum number observed and the number expected from the volume ratio of compartments, the migration was judged to be excessive. In Section 2 the difference between the minimum number and the number expected from the behavior by visual cue was tested.

What level the number of guppies finally approached was also examined by the same test between the final number observed and that expected. The median of the numbers of fish during the last 5 min of observation was used as the final number of fish. When the null hypothesis of no difference was not rejected, an approach to the expected level was admitted.

A mirror effect due to the reflection from the white paper attached on the glass walls was not observed in our experimental conditions, though, when singly put in, the guppy continued tenaciously to swim facing the glass walls.

## RESULTS

### Section I

Whether or not the initial migration is excessive in an unequal-divided tank was examined.

*Experiment 1* An opaque plastic plate was put in the center of the right compartment, so that the volume ratio of the left compartment to the right one may become 2:1 (Fig. 1).

When a population of 40 fish was introduced into the left compartment, the fish immediately migrated to the right compartment.

The number of fish declined below the balance level 27 (40×2/3) and reached 17.5. The deviation of the number 17.5 from 27 was significant ($p< 0.01$)(Table 1). Thus, the excessiveness of migration was confirmed even in an unequally divided tank.

The final number did not attain to 27 within 30

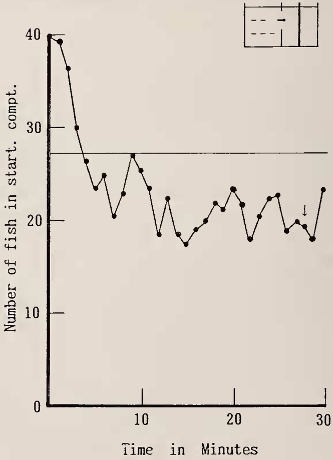


FIG. 1. Change of the number of fish in the left (starting) compartment in an aquarium, in which an opaque plastic plate was placed in the center of the right compartment. ——: a level of volume ratio of left to right compartments. ↓ indicates the median of the numbers of fish during the last 5 min of observation. For details see text.

TABLE 1. The minium number observed and final number expected from volume ratio in the left (starting) compartment in Section 1

| Expt. No. | Minimum number observed | Final number expected from volume ratio |
|---|---|---|
| Exp. 1 (Fig. 1) | 17.5 | 27 (2.7011; <0.01)** |
| Exp. 2 (Fig. 2) | 11.0 | 20 (2.1405; <0.05)* |

The number in the parenthesis represents a statistic $Z$ and significance level $p$ in the Wilcoxon one-sample signed-ranks test between the observed minimum number and the final number expected from volume ratio. * and ** represent significance at 0.05 and 0.01, respectively.

TABLE 2. The final number observed and final number expected from volume ratio in the left (starting) compartment in Section 1

| Expt. No. | Final number observed | Final number expected from volume ratio |
|---|---|---|
| Exp. 1 (Fig. 1) | 19.5 | 27 (2.4879; <0.05)* |
| Exp. 2 (Fig. 2) | 19.0 | 20 (0.2369; >0.80) |

The number in the parenthesis represents a statistic $Z$ and significance level $p$ in the Wilcoxon one-sample signed-ranks test between the observed final number and the final number expected from volume ratio. Final number observed is the median of the numbers of fish during the last 5 min of observation. For details see text. * represents significance at 0.05.

min observation ($p < 0.05$) (Table 2).

*Experiment 2* In an equal-divided tank, the number of fish in the left compartment once reached below the level of half number 20 (Fig. 2). The deviation of the minimum number of fish 11.0 from 20 was statistically significant ($p < 0.05$).

Then the population gradually approached a balance of equal number. The difference between the final number observed and 20 was not significant ($p > 0.80$).

## Section II

This section was carried out to examine whether the attainment to final balance mainly depend upon visual or non-visual cue. Under a condition that the space is apparently modified with a mirror, if the fish behave according to visual informations, the final balance would be subject to a volume ratio expected from the modified space. On the other hand, if fish react to real space, the final distribution will approach a balance expected from a real volume ratio of compartments. The expected numbers are listed in Table 4.

*Experiment 3* A large mirror was attached on

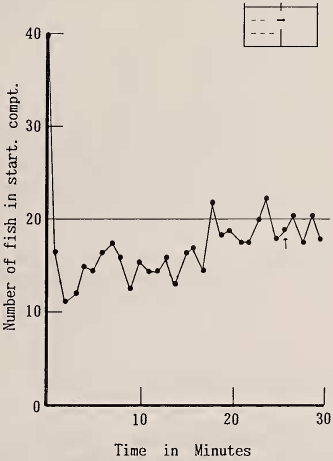
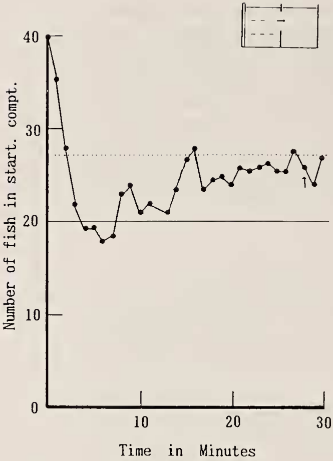


FIG. 2. Change of the number of fish in the left (starting) compartment in an equal-divided aquarium.



the left wall of the left compartment (Fig. 3). In this condition, while the compartment are spatially equal, the left (starting) compartment is visually twice as the right one.

Threat and other responses of fish to their own images in the mirror were scarcely observed. The minimum number in the left compartment was 18 (Table 3). This number differed nearly significantly at 0.05 from 27, a visually expected number.

The number of fish in the left compartment approached gradually 27 ($p>0.50$) (Table 4), not 20 expected from spatial cue ($p<0.05$).

*Experiment 4* The mirror was attached on the right wall of the right compartment (Fig. 4). The apparent volume of the right compartment is twice as the left one.

The minimum number of fish was 5 (Table 3),

Fig. 3. Change of the number of fish in the left (starting) compartment. A mirror was attached on the left wall of the left compartment. ·······: a level expected from visual cue.

Table 3. The minimum number observed and final number expected from visual cue in the left (starting) compartment in Section 2

| Expt. No. | | Minimum number observed | Final number expected from visual cue |
|---|---|---|---|
| Exp. 3 | (Fig. 3) | 18 | 27 (1.9545; ≒0.05)* |
| Exp. 4 | (Fig. 4) | 5 | 13 (2.6656; <0.01)** |
| Exp. 5 | (Fig. 5) | 10.5 | 20 (2.6502; <0.01)** |
| Exp. 6 | (Fig. 6) | 13.5 | 20 (2.0140; <0.05)* |

The number in the parenthesis represents a statistic $Z$ and significance level $p$ in the Wilcoxon one-sample signed-ranks test between the observed minimum number and the final number expected from visual cue. * and ** represent significance at 0.05 and 0.01, respectively.

Table 4. The final number observed and final numbers expected from visual and non-visual sues in the left (starting) compartment in Section 2

| Expt. No. | | Final number observed | Final number expected from non-visual cue | Final number expected from visual cue |
|---|---|---|---|---|
| Exp. 3 | (Fig. 3) | 26.0 | 20 (2.4286; <0.05)* | 27 (0.6516; >0.50) |
| Exp. 4 | (Fig. 4) | 10.5 | 20 (2.5471; <0.05)* | 13 (0.6625; >0.50) |
| Exp. 5 | (Fig. 5) | 15.5 | 27 (2.8031; <0.01)** | 20 (1.8348; >0.05) |
| Exp. 6 | (Fig. 6) | 18.5 | 27 (2.7011; <0.01)** | 20 (0.6625; >0.50) |

The number in the parenthesis represents a statistic $Z$ and significance level $p$ in the Wilcoxon one-sample signed-ranks test between the observed final number and the final number expected from visual and non-visual cues. For details see text. * and ** represent significance at 0.05 and 0.01, respectively.

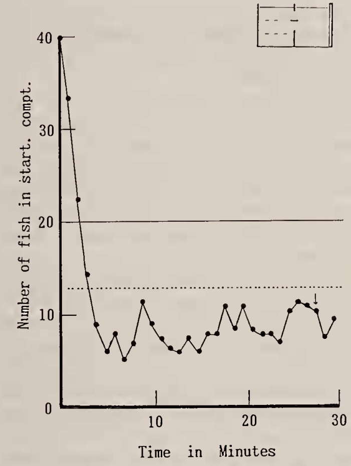


FIG. 4. Change of the number of fish in the left (starting) compartment. A mirror was attached on the right wall of the right compartment.

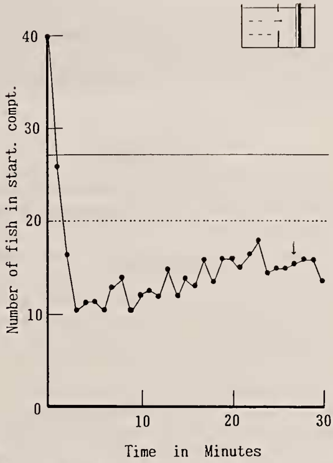


FIG. 5. Change of the number of fish in the left (starting) compartment. A mirror was placed in the center of the right compartment.

which is significantly smaller than a visually balanced number 13 ($p<0.01$).

The difference of the final number 10.5 (Table 4) from 13 was not significant ($p>0.50$), while that from the spatially expected 20 was significant ($p<0.05$).

*Experiment 5* The mirror was placed in the center of the right compartment (Fig. 5). The right compartment is visually the same as the left one, while spatially half.

The minimum number in the left was 10.5 (Table 3). The difference between 10.5 and a visually balanced number 20 was significant ($p<0.01$).

The final number 15.5 (Table 4) was not significantly different from 20 ($p>0.05$), but different from the spatially expected 27 ($p<0.01$).

*Experiment 6* A transparent glass plate was put in the center of the right compartment (Fig. 6). Here, the right compartment was visually equal to the left one, though spatially half.

The minimum number was 13.5 (Table 3), which was significantly different from the visually ex-

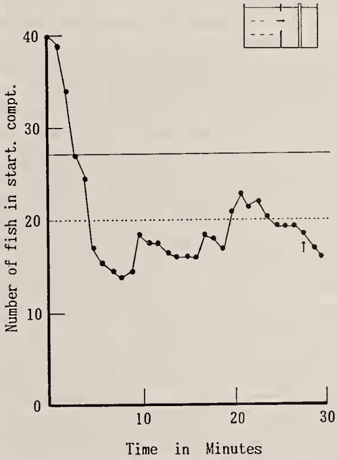


FIG. 6. Change of the number of fish in the left (starting) compartment. A transparent glass plate was placed in the center of the right compartment.

pected 20 ($p<0.05$).

The final number 18.5 (Table 4) was of non-significant difference from 20 ($p>0.50$), while significantly different from the spatially expected 27 ($p<0.01$).

## DISCUSSION

In a previous paper we concluded that the avoidance of pressure due to excessive fish density is one of the causes of E.T.M., because a guppy population confined in a small area more rapidly migrated and reached the half number level [1].

Many workers [4–8] noticed a spacing among fish individuals in the analysis of school formation since Parr's pioneer work [4]. Breder stated in 1954 that, there is a certain specified distance beyond which fish refuse to move any closer, and the regularity of spacing is brought about by an interaction of attraction and repulsion. While the attraction of fish to fish has been shown to operate by means of vision, the repulsion might work on a rather different basis. Operating at short distances, in addition to the visual component, other clues could include water movement, sound, odor and taste [5]. Symons [6] investigated the relationship between spacing and population density in the mummichog *Fundulus heteroclitus* and the stickleback *Gasterosteus aculeatus,* and reported that, when separated by 1 fish length, they spaced at random, but when within 1 fish length, they often became regularly spaced. Fenderson and Carpenter studied an interference in fish activity by the pressure due to social interaction: the salmon reared in hatchery where fish are confined at abnormally high density consumed less food than those reared in streams where population density is regulated by territorial dispersion [7]. Thus, fishes in general are apt to avoid so much pressure owing to population.

Section I obviously showed that guppy populations initially migrate excessively beyond the balance level expected from volume ratio between the left and right compartments, in either equally (Fig. 2) or unequally (Fig. 1) divided tank (Table 1).

Table 3 shows the statistical analyses of the difference between the minimum fish number and the number expected from visual cue in Section II. The initial migration of fish populations to the other compartment was significantly excessive in all the experiments.

The excessive transitory migration exhibited a large variety of time. In Section 2, in some cases the population quickly attained to the level expected from visual cue (Figs. 3 and 6), and in some cases the attainment was much retarded (Figs. 4 and 5). It is noticeable that it behaved in relation to the level expected from visual cue, not the level from non-visual cue (Table 4).

A mirror was often used in the study of animal behavior [9–14]. Lissmann [9] demonstrated that Siamese fighting fish *Betta splendens* followed the movement of a mirror turned on a horizontal axis, and Tinbergen [10, 11] showed the threatening movement by a male stickleback to its own mirror image in an extensive study dealing with visual and non-visual social releasers. Dore *et al.* [14] reported on *Betta splendens* that a mirror presentation produced a large amount of display than a conspecific placed behind the glass partition, which in turn elicited more threat than an oponent in an actual fight. The above studies indicate that fish can identify its conspecifics due to mirror images.

As to the final number of fish in the starting compartment, in Experiments 3, 4, 5 and 6 the results supported the suggestion of superiority of visual information. Statistic analysis of the difference between observed and expected values of final number of fish is shown in Table 4, which indicates that the number of fish in the left compartment finally approached the value expected from visual cue, not the value expected from space cue.

In the tank in which an opaque septum was put in the center of the right compartment, the fish were finally distributed in 2:1 as expected from the volume ratio of compartments (Fig. 1). In an equal-divided tank (Fig. 2), the distribution was 1:1. So, in either case the final distribution was due to the volume ratio of the compartments.

When the mirror is placed in the left compartment (Fig. 3), the left one is visually twice as the right one in volume; the volume ratio between two compartments is visually 2:1. The experiment actually showed the fish distribution to be 2:1.

When the mirror was put in the right compartment (Fig. 4), the final distribution was 1:2. The results mean that the fish behave according to visual information of space.

In Figure 5, a mirror was in the center of the right compartment, so the real volume ratio was 2:1, but visually 1:1. The experimental result showed that the final distribution was 1:1. Next, when a transpartent plate was put in place of the mirror, the real ratio was 2:1, but visually 1:1. The fish were finally distributed in 1:1. The above results again indicate that the fish react precisely to visual space rather than the real volume.

In Figure 4, however, there were twice number of fish in the right compartment as the left one, and accordingly the fish density was also twice. In Figure 5, as the images were in the mirror, the density of fish in the right part as a whole was twice as that of the left, while in Figure 6 the total density was the same as the left. These results suggest that the fish do not behave by recognizing their population density.

In both Figure 4 and Figure 5, however, the fish images are in the mirror. In the experiment the fish density actually was twice. Since the fish perceive the relative volume of compartments and decided their distribution, the fish must watch also the images of their own and conspecifics in the mirror. Nevertheless, they could be distributed precisely only according to the ratio of space. This contradiction could not be explained, because they perceive the space with their eyes, while they do the number of fish by the cue an other than eyes, i.e. they might be able to assess the exact number of fish individuals by sound or water vibration without deception by the mirror.

Animals in general exhibit 'curiosity (exploratory nature)' [15] to a novel environment when they are newly exposed to it. Warren and Callagan discussed the curiosity in guppies using a bowl, the bottom of which was divided to outer and inner ring areas [15]. If each animal shows a response to unknown situation, some behaviors of an animal group may be explained as a result of summation of responses of individuals, thus the curiosity may also be a cause of the E.T.M. We are now planning an analysis to examine the above possibility in comparison between a condition of closing the slit until onset of the trial and a condition of keeping fish's free passage through a slit until just before the trial. This may throw a light for further analysis of the excessive transitory migration.

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